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The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial

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SUMMARY

Background: Epidemiologic studies suggest that coffee use might protect against colorectal cancer. Inconsistencies as to the effect of coffee use and colorectal cancer between epidemiologic studies might be related to the type of coffee brew.

Objective: We studied the effect of unfiltered coffee consumption on putative biomarkers for colonic cancer risk.

Design: A total of 64 healthy volunteers (31 men and 33 women), with a mean age of 43 ± 11 years were randomly assigned to two groups in a crossover design, with two intervention periods of 2 weeks separated by a washout period of 8 weeks. Treatments were 1 L of cafetière (French press) coffee daily or no coffee. At the end of each intervention period, fasting blood samples, colorectal biopsies and 48 h faeces were collected.

Results: No effect of coffee on colorectal cell proliferation, assayed by estimating the Proliferating Cell Nuclear Antigen labelling index, was seen. Additionally, no effects were seen on the concentrations of faecal soluble bile acids and colorectal mucosal glutathione S-transferase activity. However, unfiltered coffee significantly increased the glutathione content in the colorectal mucosa by 8% and in plasma by 15%. Other aminothiols in plasma also increased on coffee.

Conclusion: Unfiltered coffee does not influence the colorectal mucosal proliferation rate, but might increase the detoxification capacity and anti-mutagenic properties in the colorectal mucosa through an increase in glutathione concentration. Whether this effect indeed contributes to a lower colon cancer risk remains to be established.

INTRODUCTION

Some 75–90% of the incidence of colon cancer is thought to be the result of environmental factors, with diet as a major determinant. Diets high in fat and low in fruits, vegetables and fibre are associated with a high cancer risk.¹ The effects of coffee on cancer risk have also been scrutinized because coffee is a widely consumed beverage. In 1991 the International Agency for Research on Cancer of the World Health Organization

concluded that the collective evidence on coffee use and colorectal cancer is compatible with a 'protective' effect.² Since then, eight epidemiological studies have further assessed coffee use in relation to colorectal cancer. The relative risk in these latest studies varied between 0.48 and 1.0, with the exception of a relative risk of 1.2 for men and 0.8 for woman in a Norwegian follow-up study on coffee use and cancer development.^{3–10} Two studies also assessed the risk of developing adenomas in relation to coffee use and reported relative risks of 0.3 and 1.0, respectively.^{4, 7} No detailed information on the type of coffee brew were reported in these studies. Overall these data reinforce the possible 'protective' effect of coffee on colorectal cancer found in earlier studies.

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In a meta-analysis of coffee consumption and the risk of colorectal cancer, three putative working mechanisms for the protective effect of coffee were discussed: (i) the anti-mutagenic properties of coffee have been related to hemicellulose fibre and polyphenolics that were identified in brewed and instant coffee; these constituents removed and destroyed mutagens in pyrolysates in food;¹² (ii) the influence on colonic motility;¹¹ and (iii) the influence on faecal bile acids—coffee might reduce the hepatic excretion of bile acids and thereby lower the risk for colon cancer.^{13–15} Secondary bile acids are promoters of colon carcinogenesis in laboratory animals, although human data are less convincing.^{16–19} Epidemiological studies have shown a positive correlation between faecal bile acid concentration and colon cancer risk.²⁰ Furthermore, colonic bile acids have cytotoxic properties and can stimulate the proliferation of colonic cells.^{21, 22} Measurement of the effect of coffee on faecal bile acids might thus be useful in evaluating the epidemiological findings on coffee and colon cancer.

A marker that might be closer to the actual process of carcinogenesis is colonic cell hyperproliferation which is thought to be an early event in the multi-step process of the colonic adenocarcinoma sequence.²³ Measurement of colorectal mucosal cell proliferation parameters, such as the labelling index and the distribution of the proliferating cells in the crypt, have been used as endpoints in clinical trials.²⁴ The colorectal mucosal cell proliferation can be reduced or normalized by possible chemopreventive agents such as calcium, resistant starch, vitamin A, C and E, and ω -3 fatty acids.^{22, 25–27}

Animal studies on the effect of coffee in the digestive tract have focused on the effect of coffee oil and its major non-triacylglycerol constituents, the diterpenes cafestol and kahweol. Coffee oil reduced the frequency of 1,2-dimethylhydrazine induced adenocarcinomas of the colon in rats whereas these diterpenes enhanced the glutathione S-transferase activities of small intestine and liver in mice and rats.^{28–31}

The glutathione S-transferase biotransformation system is involved in the liver metabolism and detoxification of many xenobiotics. These enzymes are also present in the human gastrointestinal tract.³² Dietary components which increase their concentrations in the gastrointestinal tract are thought to increase its detoxifying capacity and might help to prevent cancer.^{33–36}

The inconsistencies between the observational studies suggest that either not all types of coffee brew have the same effect on the colorectal cancer risk or that the

effect is spurious. In addition, the mechanism for achieving colon cancer prevention is still unclear. We therefore studied the effect of coffee on putative biomarkers for colonic cancer in human volunteers. We used unfiltered coffee so as to include all possible components, such as the coffee diterpenes.³⁷

METHODS

Subjects and design

Our primary hypothesis was that coffee intake would decrease the proliferating cell nuclear antigen labelling index in the colorectal mucosa. Accessory hypotheses were that coffee would increase the glutathione S-transferase enzyme activity and that the concentration of glutathione in the colorectal mucosa and would decrease soluble faecal bile acids. To test this we performed a comparative crossover study using unfiltered coffee in healthy volunteers. The study protocol was approved by the local human ethics committee.

We recruited the healthy volunteers through an article in a local paper and posters on the hospital and university grounds. Subjects were eligible if they: drank an average of 2–3 cups of filtered coffee per day; were between 24 and 70 years of age, were not following a medical diet; used no laxatives, non-steroidal anti-inflammatory drugs, vitamin supplements or lipid-reducing drugs; and had not used antibiotics within 2 months before entering the study. Exclusion criteria were: a history of liver or kidney diseases (serum alanine aminotransferase activity greater than 30 U/L or a serum creatinine concentration above the upper limit of normal); a history of cholecystectomy or partial bowel resection; a serum total cholesterol greater than 6.5 mmol/L or fasting triacylglycerol greater than 3.0 mmol/L; or living more than 50 km from the University site.

Seventy volunteers were eligible and gave their written informed consent. They were stratified by gender and smoking habit and then randomly assigned to the two intervention sequences. Subjects were enrolled in three shifts; one shift started in April, one in June and one in September of 1997.

Subjects were instructed to maintain their usual diet during the study. We checked compliance with a 3 days dietary recall in each intervention period. Diet composition was calculated using the Dutch nutrient database.³⁸

The study consisted of two intervention periods of 2 weeks each separated by a washout period of 8 weeks, in a crossover design. One group started on 1 L per day of unfiltered cafetière coffee, which is also known as French Press or plunger coffee. One litre of coffee equals six large cups. The other group did not use coffee, but instead used water, milk, chocolate drink, tea or broth. Each of these beverages was limited to a maximum of three cups per day. On day 13 and 14 of each intervention period, 48 h faeces were collected on dry ice, and on day 15, fasting blood-samples and colorectal biopsies were taken. During the second period treatments were switched. All samples were coded so as to hide the identity and treatment of subjects to laboratory personnel. For each individual the samples obtained in the two intervention periods were analysed simultaneously.

Coffee preparation

We used 'Douwe Egberts' brand, coarsely ground coffee (Sara Lee Company, Utrecht, the Netherlands), consisting of a blend of arabica and robusta beans. This is the most widely used coffee brand and blend in the Netherlands. We packaged the coffee in evacuated plastic bags in daily portions in order to preserve the coffee aroma. Volunteers put 39 g of ground coffee into a 1-L cafetière coffee-pot (Blokker, Amsterdam, the Netherlands); 600 mL of boiling water was poured on to the grounds as described.³⁷ Subjects then stirred the brew and after 5 min they pushed down the plunger to separate the brew from the grounds. This resulted in 500 mL of cafetière coffee. The mean (\pm s.d.) cafestol concentration was 34 ± 3 mg/L and the kahweol concentration was 26 ± 1 mg/L. Subjects prepared and consumed two such 500-mL portions daily. We advised them to drink the coffee throughout the whole day and provided the volunteers with insulated flasks. We considered it highly likely that the subjects were compliant because few would enjoy drinking a large amount of strong coffee all at once.

Colonic tissue and assays

Colorectal mucosal biopsies (six biopsies per location) of normal tissue at 10–15 cm from the anal verge were obtained during sigmoidoscopy without previous bowel preparation. Three biopsies were immediately stretched and fixated in ethanol (70% v/v) and consecutively

embedded in paraffin and stored until use for immunohistochemical proliferating cell nuclear antigen analysis. The remaining three biopsies were immediately frozen in liquid nitrogen and stored at -80°C until use for glutathione S-transferase phenotyping and glutathione assay.

Proliferating cell nuclear antigen labelling. Standard histological sections of the colorectal biopsies embedded in paraffin were prepared on slides coated with poly L-lysine (Sigma). Sections were incubated with PC10 antibodies (Dako, Glostrup, Denmark) diluted 1:400 in phosphate buffered saline (PBS) containing 4% bovine serum albumin for 1 h at 25°C , after de-paraffinization and blocking of endogenous peroxidase activities, as described before.²² The binding of the PC10 antibody was visualized with a streptavidin–biotin system (Histostain-SP, Zymed Laboratories, San Francisco, USA). Ten complete longitudinally sectioned crypts (20 crypt-halves) were scored with a $40\times$ objective. All cells with a homogeneous and moderately or strongly stained nucleus were counted as positive. Epithelial cell proliferation was expressed as the total labelling index, which is defined as the fraction of labelled cells in the whole crypt. In addition, the crypts were divided into five compartments to analyse the spreading of labelling throughout the crypt. The intra- and inter-observer correlation for estimating the labelling index was 0.976 and 0.926, respectively.

Glutathione S-transferases and glutathione content. The frozen biopsies were homogenized in a glass/glass Potter–Elvehjem tube after dilution with a buffer solution (0.25 M saccharose, 20 mM Tris, 1 mM dithiothreitol, pH 7.4) as described before.³³ Aliquots of post-centrifugation supernatant, representing the cytosolic fractions, were frozen in liquid nitrogen and stored at -80°C until use. Protein concentrations were determined in duplicate according to the method of Lowry *et al.*³⁹ Total glutathione S-transferase enzyme activity was assayed in duplicate with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to Habig *et al.*⁴⁰ Total glutathione was quantified in duplicate by high performance liquid chromatography after reaction with monobromobimane according to Fahey and Newton, with minor modifications, as described before.⁴¹ Glutathione S-transferase isoenzyme concentrations were determined as follows: cytosolic samples were subjected to sodium dodecyl sulphate-polyacrylamide

gel and subsequently to Western blotting.⁴² Western blots were stained with specific antibodies against glutathione S-transferase Alpha, Mu and Pi, and subsequently quantified by densitometry. The within-assay and between-assay variation were both $\pm 5\%$ for glutathione S-transferase Mu and 25% for glutathione S-transferase Alpha and Pi. Class Alpha antibodies react against glutathione S-transferase A1-1, glutathione S-transferase A1-2 and glutathione S-transferase A2-2, class Pi antibodies recognize glutathione S-transferase P1-1 and class Mu antibodies are directed against glutathione S-transferase M1a-1a, glutathione S-transferase M1a-1b and glutathione S-transferase M1b-1b.^{42–44}

Blood samples and assays

Fasting venous blood samples were collected on day 15 of each intervention period.

Serum cholesterol and alanine aminotransferase were analysed in the routine clinical laboratory of our hospital according to standard clinical chemical procedures at 37 °C on a Hitachi 747 analyser (Roche Boehringer Mannheim, Almere, the Netherlands). EDTA-treated blood samples for analysis of glutathione, cysteine, cysteinylglycine and total homocysteine were immediately placed on ice until the plasma was separated by centrifugation. EDTA-treated plasma samples were stored at –20 °C until analysis. Concentrations of glutathione, cysteine, cysteinylglycine and total homocysteine in plasma were determined by automated reduction with NaBH₄/dithiothreitol and derivitized by Thiolyte (Calbiochem, La Jolla, USA) as described previously.⁴⁵ The inter- and intra-assay variation was less than 5%.

Faeces

Forty-eight hour faeces were collected on dry ice, weighted and subsequently stored at –20 °C until processing. The thawed faeces was homogenized with a blender and a 5-g sample was freeze dried for 48 h (Hetosicc, Allerød, Denmark). The dry weight is the freeze dried weight as a percentage of the wet weight.

Faecal water. Faecal water was prepared as described previously with slight modifications.²² The homogenized faecal samples were centrifuged at 40 000 *g* for 2 h at 4 °C. Faecal water was removed and filtered

using a 5- μ m filter (Schleicher & Schuell, Dassel, Germany) and stored at –20 °C until analysis.

Bile acid analysis. Bile acids were determined as previously described.²² In short, after enzymatic hydrolysis the bile acids were extracted from faecal water by SepPak C18 chromatography. The saponifiable conjugates were then hydrolysed at 60 °C for 2 h in 1 mL of 1 M KOH solution in methanol. After a second extraction by SepPak C18 chromatography, the unconjugated bile acids were separated from the neutral sterols by Lipidex DEAP chromatography and measured after methylation and silylation by capillary gas chromatography (column CP Sil 5CB) on a Packard 430 gas chromatograph.

Statistics

We designed the study to pick up an effect of coffee on the proliferating cell nuclear antigen labelling index as a proliferation biomarker in normal colorectal mucosa. To pick up an effect of 10% at $P < 0.05$ with a power of 80% in a crossover design we required 56 subjects.

The wet and dry weight of the faeces and the faecal soluble bile acids were analysed. The following biomarkers were investigated in the colonic mucosa: the proliferating cell nuclear antigen labelling index, the glutathione S-transferase enzyme activity and glutathione S-transferase Alpha, Pi and Mu isoenzyme contents, as well as the glutathione content. The glutathione, cysteine, cysteinylglycine and homocysteine concentrations in plasma and the cholesterol and alanine aminotransferase concentrations in serum were investigated. Differences in biomarkers between the end of the no-coffee period and the end of the coffee period were calculated per subject and analysed by using a two-sided unpaired *t*-test (SPSS Inc, Chicago, USA).

RESULTS

A total of 64 volunteers (31 men and 33 women) with a mean age of 43 ± 11 years (range: 24–70 years) completed the study. Six volunteers dropped out; one subject stopped due to palpitations and tremor during the first days of drinking the cafetière coffee; the other five subjects resigned for reasons not related to the study. The characteristics of the 64 volunteers who completed the study are shown in Table 1.

Table 1. Baseline characteristics of the healthy volunteers who took part in this comparative crossover study of the influence of unfiltered coffee on biomarkers for colonic cancer

Total no. volunteers	64
No. men:women	31:33
No. smokers	16
Age (years \pm s.e.)	43.4 \pm 1
Age range (years)	24–70
Body-mass index (kg/m ²)	24.5 \pm 0.5
Cholesterol (mmol/L)	5.0 \pm 0.1
Triacylglycerol (mmol/L)	1.0 \pm 0.1
Alanine aminotransferase (U/L)	13.0 \pm 0.6
Creatinine (μ mol/L)	86.8 \pm 1.4

During the coffee period, in which subjects drank 1 L of coffee daily, more water (mean 361 g) was consumed than in the no-coffee period (Table 2). The protein intake in the coffee period was 8 g higher than in the no-coffee period. No other differences in reported dietary intakes were noticed.

Coffee consumption did not affect the total proliferating cell nuclear antigen-labelling index, the faecal soluble bile acid concentrations, the colorectal glutathione S-transferase enzyme activity, the glutathione S-transferase Alpha, Mu and Pi isoenzyme concentrations or the faecal wet and dry weights (Table 3). Additionally, no differences in the five compartmental proliferating cell nuclear antigen-labelling indexes were seen (data not shown).

However, the mean colorectal mucosal glutathione concentration was 30.1 nmol/mg protein at the end of the no-coffee period and 32.5 nmol/mg protein at the end of the coffee period (Table 3). Plasma glutathione increased from 5.5 μ mol/L at the end of the no-coffee period to 6.3 μ mol/L at the end of the coffee period

(Table 4). We thus observed an increase in colorectal mucosal glutathione concentration of 8% or 2.5 nmol/mg protein ($P = 0.01$) and in plasma glutathione concentration of 15% or 0.8 μ mol/L ($P = 0.003$) caused by unfiltered cafetière coffee. The effect of coffee on the plasma and colorectal mucosal glutathione concentrations were seen in both treatment sequences (data not shown).

The concentrations of other thiols in plasma also increased significantly on coffee (Table 4): cysteinylglycine by 9% or 4.2 μ mol/L ($P = 0.004$), cysteine by 3% or 7.7 μ mol/L ($P = 0.004$) and homocysteine by 10% or 12.8 μ mol/L ($P = 0.001$).

As expected, consumption of unfiltered coffee increased mean serum cholesterol by 10% or 0.5 mmol/L (95% CI: 0.3–0.6, $P < 0.001$) and serum alanine aminotransferase activity by 2.5 U/L (95% CI: 1.0–3.9, $P = 0.001$).³⁷ In three of the subjects the alanine aminotransferase activity exceeded the upper normal reference level after 2 weeks of drinking coffee; in one subject this was observed at the end of the no-coffee period. Alanine aminotransferase activity returned to normal within 2–3 weeks in all but one subject, in whom values were normalized only after 9 months (data not shown).

DISCUSSION

We found that drinking 1 L of unfiltered coffee every day for 2 weeks did not affect the colorectal cell proliferation. The crossover design of our study and the distribution of subjects over three shifts eliminate chance fluctuations and seasonal influences as confounding factors. These results thus do not reinforce the hypothesis that coffee consumption reduces the risk of

Table 2. Average dietary intakes of the 64 healthy volunteers who took part in this comparative crossover study comparing a 1 L coffee period with a no-coffee period

	No coffee period	Coffee period	Difference	95% CI of difference
Energy (MJ/day)	10.1	10.1	0.1	–0.5 to 0.5
Fat (g/day)	96	97	1	–6 to 9
Protein (g/day)	85	93	8†	2 to 13
Carbohydrates (g/day)	278	270	–8	–23 to 7
Fibre (g/day)	19	19	0	–2 to 1
Water (g/day)*	2519	2880	361†	195 to 527

CI, confidence interval.

Three-day dietary recall data were collected in both intervention periods.

*The amount of water included beverages as well as the water in solid foods.

† $P < 0.05$.

Table 3. Faecal and colorectal mucosal biomarkers for colonic cancer in the volunteers receiving 1 L unfiltered coffee or no-coffee, daily, for 2 weeks each, in this crossover study

	Number of subjects	No-coffee period mean \pm s.d.	Coffee period mean \pm s.d.	Difference	95% CI of difference
Faeces					
wet weight (g/48 h)	64	323 \pm 159	337 \pm 184	14	-28 to 58
dry weight (%)	64	25 \pm 5	24 \pm 4	-1	-2 to 1
Soluble faecal bile acids in faecal water					
total (μ mol/L)	63	170 \pm 207	138 \pm 117	-32	-83 to 17
secondary (μ mol/L)	63	126 \pm 126	111 \pm 103	-15	-39 to 9
Colorectal mucosa					
Glutathione S-transferase biotransformation system					
GST activity (nmol/min/mg protein)	62	211 \pm 67	210 \pm 70	-1.0	-23 to 20
GST isoenzymes (ng/mg protein)					
GST Alpha	59	30.2 \pm 9.0	34.5 \pm 10.1	4.3	-4.1 to 12.7
GST Mu	60	618 \pm 98	644 \pm 104	26	-62 to 116
GST Pi	59	2017 \pm 59	2157 \pm 124	140	-98 to 377
Glutathione (nmol/mg protein)	62	30.1 \pm 6.6	32.5 \pm 6.5	2.5*	0.5 to 4.4
Cell proliferation					
PCNA-labelling index (%)	58	21.0 \pm 1.2	19.7 \pm 1.1	-1.2	-4.0 to 1.5

Number of subjects: due to lack of sufficient material not all assays could be performed on all 64 subjects.

CI, confidence interval; GST, glutathione S-transferase; PCNA, proliferating cell nuclear antigen.

* $P = 0.01$.

Table 4. Plasma thiols in volunteers receiving 1 L of unfiltered coffee or no-coffee, daily, for 2 weeks each, in this crossover study

	Number of subjects	No-coffee period Mean \pm s.d.	Coffee period Mean \pm s.d.	Difference	95% CI of difference
Glutathione (μ mol/L)	45	5.5 \pm 1.8	6.3 \pm 1.4	0.8†	0.3 to 1.4
Cysteinylglycine (μ mol/L)	63	46.8 \pm 7.9	51.0 \pm 8.3	4.2‡	1.4 to 6.9
Cysteine (μ mol/L)	63	209.8 \pm 23.4	217.5 \pm 21.8	7.7‡	2.5 to 13.0
Homocysteine (μ mol/L)	63	12.8 \pm 3.3	14.0 \pm 3.5	1.2*	0.5 to 1.9

CI, confidence interval.

* $P = 0.001$; † $P = 0.003$; ‡ $P = 0.004$.

colon cancer. However, by themselves our findings on proliferation are insufficient to refute the epidemiological findings on coffee and prevention of colon cancer. Our findings might also indicate that the influence of coffee on colon cancer risk might act through pathways other than those influencing the cell proliferation rate. Colorectal cancer may arise when the balance between cell proliferation and apoptosis is disrupted in favour of uncontrolled growth.⁴⁶ Most research on colon cancer has focused on the molecular regulators of cell-cycle progression and proliferation, but defective apoptosis may also be involved.⁴⁷ Therefore, factors which influence colonic carcinogenesis in a beneficial way, might not have an effect on colonic cell proliferation.⁴⁸

An alternative explanation for the lack of effect on proliferating cell nuclear antigens could be that it might be difficult to (further) decrease the 'normal' cell proliferation rate in healthy volunteers. However, we found a wide range of proliferating cell nuclear antigen values in these normal subjects, and therefore we find this explanation unlikely.

Secondary faecal soluble bile acids which can stimulate colonic cell proliferation, were also investigated. The accessory hypothesis that we tested was that unfiltered coffee decreases the faecal soluble bile acid concentration. In line with the results on colorectal mucosal proliferating cell nuclear antigen labelling index, the total faecal soluble bile acid concentration as well as the

cytotoxic secondary faecal soluble bile acid concentration did not change between the intervention periods (Table 3). Therefore, the cytotoxic secondary bile acids do not seem to be involved in the putative protective effect of coffee in colonic cancer development.

The hypotheses that coffee would increase the glutathione S-transferase activity and the glutathione concentration in the colorectal mucosa were partly confirmed. Animal studies have found that coffee oil and its constituents, the diterpenes cafestol and kahweol, induced intestinal glutathione S-transferase enzyme activity; glutathione content has not been investigated.^{29–31} The amount of coffee constituents fed in animal studies was much higher than can be achieved in humans drinking coffee. This might explain why we did not find an induction of glutathione S-transferase enzyme activity or glutathione S-transferase isoenzyme concentrations in the colorectal mucosa. However, unfiltered coffee did increase colorectal glutathione concentration by 8% and plasma glutathione concentration by 15% (Tables 3 and 4). It is unlikely that this was caused by the slightly higher protein intake during the coffee period (Table 2). We are not aware of experimental data on the effect of protein intake on plasma glutathione concentrations in man, but earlier findings on the relation between protein intake and plasma homocysteine concentrations show that the effect of small differences in protein intake are negligible.^{49–51} We therefore assume that the rises in glutathione and other thiols in plasma (Table 4) were also caused by substances in the coffee rather than by minor differences in protein intake.⁵²

Normal colonic epithelium is a rapidly proliferating tissue because renewal of the colonic epithelium takes place every 4–6 days. Such rapidly proliferating cells are more sensitive to toxic compounds and mutagenic changes and therefore to malignant transformation, which is in essence a specific series of mutations.⁵³ Glutathione might inhibit this process through several mechanisms. First, intracellular glutathione protects the cell from DNA damage by mutagenic compounds and xenobiotics. This detoxifying capacity involves enzymatic as well as non-enzymatic processes. Glutathione reacts non-enzymatically with some mutagenic compounds such as hydroxyl radicals, but requires glutathione S-transferase enzymatic catalysis to detoxify reactive electrophilic xenobiotic.^{54–58} The newly formed compounds are more water soluble and less toxic than the original compound and can be excreted more easily

through the bile and urine.⁵⁹ This is confirmed by studies in cultured human lymphoblastoid cells, in which the frequency of both spontaneous and long wavelength ultraviolet-induced mutations at the hypoxanthine guanine phosphoribosyl transferase locus can be enhanced at least fivefold by depletion of glutathione. Reactive intermediates, such as toxic compounds and xenobiotics, normally quenched out by glutathione, appeared to be partly responsible for this effect.⁶⁰

A second mechanism may involve DNA repair. A decrease in glutathione concentration by buthionine sulfoximine, a specific inhibitor of γ -glutamylcysteine synthetase, inhibited DNA repair in cisplatin-resistant human ovarian cancer cells. Treatment of cells with glutathione esters after buthionine sulfoximine resulted in a complete recovery of DNA repair enzyme activity.⁶¹ Furthermore, glutathione is a cofactor in the synthesis of DNA precursors, especially the deoxyribonucleotide triphosphates.^{62, 63}

A third potential working mechanism of glutathione lies in the possible role of mitochondrial glutathione homeostasis in the direct regulation of apoptosis.^{64–66}

Thus there are a number of mechanisms that may link the rise in colorectal mucosal glutathione observed by us with the epidemiologically observed inverse association between coffee intake and colon cancer. One important question that remains is whether the effect on glutathione and the other thiol components was caused by substances present only in unfiltered coffee such as used by us, or by factors that are also present in filtered coffee. Future studies may answer this question.

Coffee induces the motor activity in the colon in 60% of healthy volunteers.⁶⁷ The magnitude of this motor activity induction by 240 mL coffee is similar to that of a meal.⁶⁸ The speed of the response, within 4 min of ingestion, suggests that coffee may induce a 'gastrocolonic' response.^{11, 67} Colonic motility could be related to colonic cancer risk by the influence on the exposure of the epithelia to colonic contents. We did not find changes in the wet and dry weight on coffee. Therefore we conclude that an increased motility on coffee seems not to be responsible for the putative protective effect of coffee in colonic cancer development.

Coffee consumption in most populations varies from one to seven cups per day which is less than the six 'large' and strong cups used in our study.⁶⁹ Thus our results only apply to heavy coffee drinkers.

We conclude that unfiltered coffee does not influence the colorectal mucosal proliferation rate, but intake of

large amounts might locally increase the detoxification capacity and anti-mutagenic properties in the colorectal mucosa through an increase in glutathione concentration. Whether this effect indeed contributes to a lower colon cancer risk remains to be established.

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